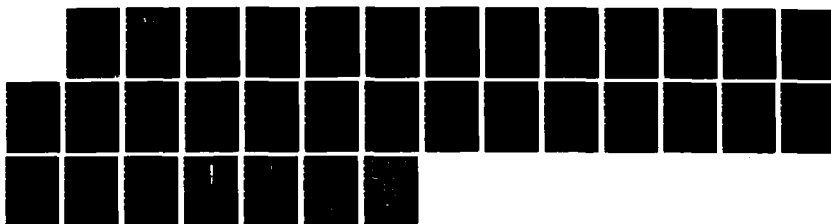AD-A192 597   HYPOTHALAMIC-PITUITARY-ADRENAL RESPONSES TO SHORT   1/1
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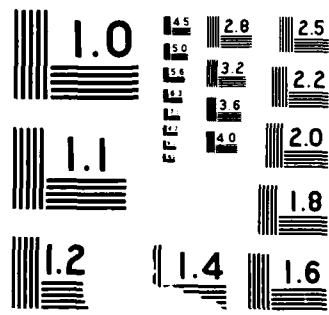UNCLASSIFIED   JAN 88 USARIEM-M-16/88   F/G 6/1   NL

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ABSTRACT

Beta-endorphin (β-EP), adrenocorticotropin (ACTH) and cortisol plasma concentrations were examined before and after maximal exercise at four intensities (36, 55, 73 and 100% of maximal leg power [MLP]) utilizing a computerized cycle ergometer. All intensities were greater than those eliciting peak oxygen uptake for the individual subjects. Blood samples were collected at rest, immediately following exercise (IP) and at 5 and 15 min post-exercise. Significant ($p < 0.05$) increases were observed at 36% MLP for β-EP and ACTH at IP, 5 and 15 min post-exercise. Plasma cortisol increased at 36% MLP at 15 min post exercise. Blood lactate significantly increased at all post-exercise collection points for exercise intensities of 36, 55 and 73% MLP and at 5 min post exercise for 100% MLP. β-EP concentrations at 36% MLP were significantly correlated ($r = 0.75$) with capillary density (mm^{-2}) and cortisol concentrations at 36% MLP were significantly correlated ($r = 0.89$) with % type II muscle fibers. No other significant relationships were observed. These data show that brief, high intensity exercise results in a non-linear exercise response pattern when increasing levels of power output up to maximal are utilized. Furthermore, blood lactate levels do not appear to influence hypothalamic-pituitary-adrenal responses to very high intensity exercise. Our observations also suggest that skeletal muscle morphology may influence hypothalamic-pituitary-adrenal responses to this exercise.

Key words: Beta-endorphin, adrenocorticotrophin, cortisol, blood lactate, skeletal muscle fiber morphology, anaerobic exercise.

A wide variety of studies have demonstrated that endurance exercise (i.e. submaximal to peak oxygen uptake) activates the hypothalamic-pituitary-adrenal axis. This is evidenced by significant increases in plasma concentrations of Beta-endorphin(β-EP), adrenocorticotropin (ACTH) and cortisol (7, 9, 11, 12, 14, 17, 18, 19, 22, 24, 29, 31, 32, 34, 36, 39).

Recently, greater attention has been focused on plasma alterations of these hormones following high intensity exercise described as ranging from approximately 110-156% of that which elicits maximal oxygen consumption (6,12,19,29,33). Significant increases have been observed post-exercise for β-EP, ACTH, and cortisol following this type of exercise. The question remains as to whether exercise at even higher power outputs induces any changes in these plasma hormone concentrations. Furthermore, to our knowledge, no attempt has ever been made to examine the hormonal responses over a wide range of exercise intensities at very high power outputs. Thus, the primary purpose of this study was to examine plasma alterations of β-EP, ACTH and cortisol to four exercise intensities of high power output. A secondary purpose was to examine the possible influence of skeletal muscle fiber morphology on hormonal responses observed. Such data should enhance the understanding of how the hypothalamic-pituitary-adrenal axis responds to high intensity exercise and the possible relationship to skeletal muscle tissue morphology.

METHODS

Subjects. Ten normally active, healthy male subjects gave written informed consent to participate in this study. Each volunteer was medically screened

by a physician, was taking no medications and had no history of endocrine disorders. A description of the subjects physical characteristics is presented in Table 1.

Each subject reported to the laboratory several times for familiarization and preliminary testing. Subjects' height, weight and body composition were determined in one laboratory session after an overnight fast. Body composition was determined by standard hydrostatic weighing methodology using a load cell interfaced with a Hewlett Packard desk top computer (21,25). Residual lung volume was determined using the oxygen dilution method described by Wilmore et al.(47).

Maximal oxygen consumption (VO_2max) was determined using a discontinuous protocol previously described (35). The cycle ergometer employed was the same as that used in determining maximal leg power. During all testing oxygen consumption and cardio-respiratory data were obtained from an on-line metabolic system previously described (10). Heart rate was monitored via ECG using a lead II configuration.

Muscle Biopsy Procedures and Analyses. Muscle biopsy samples were obtained approximately one week prior to testing from the superficial portion of the vastus lateralis muscle of the dominant leg utilizing the percutaneous needle biopsy technique of Bergstrom(4) as modified by Evans et al.(15). Special attention was taken to approximate the same biopsy location in all subjects using a depth of approximately 2 cm. Repeat biopsies (randomly performed) demonstrated non-systematic and insignificant interbiopsy variations in fiber-type distribution.

The muscle samples were oriented, placed in embedding medium, and frozen in isopentane cooled to -160°C with liquid nitrogen and stored at -120°C until analyzed. Serial cross sections (12μm thick) were cut on a cryostat (American Optical, Buffalo, N.Y.) at -20°C and placed on glass coverslips for subsequent histochemical analysis.

The histochemical analysis used for fiber typing consisted of assaying for myofibrillar ATPase activity at pH 4.3, 4.6, and 10.3 (5,42,43). Muscle fiber types were divided into three groups (types I, IIA and IIB) based on the stability of their ATPase activity in the preincubation medium (42,43).

The percentage of each fiber type was calculated from sections containing an average of 935 (range 389 to 1732) fibers. Calculations of the various fiber type percentages were computed by a Zeiss Interactive Digital Analysis System (ZIDAS) (Carl Zeiss, West Germany). This involved projection of the cross-sections at a constant magnification with a Zeiss microscope (Standard 16 with drawing tube, Carl Zeiss, West Germany) onto a digitizing tablet with self contained computer (containing appropriate morphometric programs) interfaced with a main frame computer (VAX 11/780, Digital Equipment Corporation, Maynard, MA) used for immediate data storage, and analysis.

Muscle fiber areas were determined using NADH-tetrazolium reductase stained fibers (37). The perimeter of all intact fibers of each section for Type I and Type II were measured. Cross-sections were again projected at constant magnification with a Zeiss microscope (Standard 16 with drawing tube) onto a digitizing tablet. Fiber area was determined by tracing the perimeter of all fibers of each type on this digitizing tablet and the areas were computed by

the ZIDAS system. The relative muscle area occupied by Type II muscle fibers was calculated according to a formula described elsewhere (45,46).

Capillary density (cap•mm^{-2}) and capillaries per fiber (cap•Fib^{-1}) were determined from amylase-periodic acid-Schiff stained fibers and analyzed by methods previously described in detail (1,2).

Maximal Leg Power and Relative Exercise Intensity Determinations. Maximal leg power (MLP) was determined using a cycle ergometer and computerized data collection/processing system and test protocol previously described (27,28,30). Each subject was tested for maximal power for one-revolution (i.e. highest score of five revolutions) on the cycle ergometer at 60 rpm. Three consecutive tests with a minimum of 20 minutes rest between tests were performed. MLP was operationally defined as the mean of the highest two scores of the three tests to avoid the influence of an aberrant test result. The subject was seated behind the crank in a rigid metal armchair. This position was used since the high force during maximal power pedalling results in the subject rising off a standard bicycle saddle. The distance from the chair to pedal crank was set for each subject and kept constant for all testing.

The MLP (100%) and three exercise intensities relative to MLP (36, 55, and 73%)(approx. 107, 144, 219 and 325% of $\dot{V}O_2$max, respectively) were used in this investigation to evaluate the effects of different high intensity exercise stress on plasma hormone concentrations. Preliminary testing demonstrated the test re-test reliability (r) of the 36, 55, and 73% MLP tests to be 0.92, 0.95, and 0.96, respectively.

Each test involved setting cycle resistance at the % of MLP at which the subject was to exercise. The pedal crank was then run at 60 rpm by the motor and the subject moved his legs freely without trying to push against the pedals. An investigator orally signalled the subject to begin pedalling at the same time as the ergometer motor reduced the pedal crank rpm by 10%. The subject had to generate power at a level predetermined by the investigator in order to maintain ergometer speed at 60 rpm. An analog panel meter in front of the subject indicated whether speed was being maintained. An audible electric metronome set at 120 beats·min^{-1} gave the subject additional assistance in maintaining pedalling cadence. Warnings were given when rpm's dropped. When the subject experienced extreme difficulty maintaining pedalling speed, he was verbally encouraged to continue. The test ended when a subject dropped more than three percent below the set speed for seven seconds. The seven seconds were subtracted from the total time of exercise.

Blood Collection Test Procedures. Each subject performed the four exercise intensities in a random order. Testing was conducted from 0800 to 1000 hr and each subject was tested at the identical time each day. Prior to the test, subjects refrained from food for 8 hr and exercise for 24 hr. Prior to each exercise test, a 20 gauge teflon cannula was placed into an antecubital arm vein. The cannula was kept patent with a continuous flow of isotonic saline. After the cannula was inserted, subjects rested in the seated position and two pre-exercise resting blood samples (R_1 and R_2) were obtained 20 minutes apart. Blood samples were also obtained immediately following each exercise bout to exhaustion and at 5 and 15 minutes post-exercise. Blood was collected and gently mixed in pre-cooled plastic syringes containing appropriate

preservatives (EDTA, 7.2mg/5ml whole blood). Blood to be used for subsequent
radioimmunoassay (RIA) was centrifuged at 2000 x g at 4^0C for 15 min and
plasma was immediately frozen to -120^0C. Samples were thawed only once for
analysis which occurred within 8 wk.

Biochemical Analyses. Plasma β-EP, ACTH and cortisol immunoreactivity
values were determined using RIA procedures. All samples (in duplicate) for
each RIA were measured in the same assay to avoid run-to-run assay variations.
Determinations of different plasma immunoreactivity values were accomplished
with the use of a Beckman 5500 Gamma counter and on-line data reduction
system. The RIA used for plasma β-EP has been previously described in detail
(31). Cross reactivity with Beta-lipotropin is less than 5% . The intra-
assay coefficient of variation was 3.0%. Plasma ACTH was measured by a double
antibody RIA (Diagnostic Products Corp., Los Angeles, CA) sensitive to 1.54
pmol•L^{-1}. Intra-assay coefficient of variation was 3.7%. Plasma cortisol was
measured using a solid phase RIA (Diagnostic Products Corp., Los Angeles, CA)
and was sensitive to 8.27 nmol•L^{-1}. The intra-assay coefficient of variation
was 3.4%. Hemoglobin was analyzed in triplicate using the cyanmethemoglobin
method (Sigma Chemical Co., St. Louis, MO) and hematocrit was analyzed in
triplicate using a standard micro-capillary technique. Changes in plasma
volume,pre-to immediately post-exercise were calculated from changes in
hematocrit and hemoglobin (13). Blood lactate was analyzed in triplicate
using a micro blood Lactate Analyzer - 640 (Wolverine Medical, Alto, MI).

Statistical Analyses. Statistical evaluation of the data was accomplished
by using an analysis of variance (ANOVA) with repeated measures and subsequent
Tukey post-hoc tests. For analysis of plasma responses, the baseline was

quantified as the mean response of the two pre-exercise values. Pearson product-moment correlation coefficients were calculated for the entire data set. Simple and multiple regression analyses were also utilized to examine selected variable relationships. Statistical significance was chosen as $p < 0.05$.

Results

A description of the physiological responses at each exercise intensity (%MLP) and maximal oxygen consumption utilizing the cycle ergometer is presented in Table 2. The results of the muscle fiber analysis are presented in Table 3.

No significant differences were observed between R_1 and R_2 baseline hormonal measurements. The plasma responses of β-EP to the different exercise intensities are shown in Figure 1. Significant increases above rest were observed only after the 36% MLP exercise intensity. These increases were seen immediately post, 5 and 15 min following exercise.

The plasma responses of ACTH (see Figure 2) followed a pattern similar to β-EP with significant increases occurring only after the 36% MLP exercise intensity. Again the increases were observed at each measurement time point during the post-exercise period.

The plasma responses of cortisol are presented in Figure 3. The only significant increase observed was at 15 min of recovery following the 36% MLP exercise intensity.

Blood lactate changes are shown in Figure 4. Significant increases were evident at all measurement time points for exercise intensities of 36, 55 and 73% MLP. At 100% a significant increase was observed at 5 min post-exercise.

Changes in mean plasma volume ($\pm$1 SE) were: 36% MLP, -10.3% ($\pm$2.8); 55% MLP, -4.5% ($\pm$2.9); 73% MLP, 0.35($\pm$2.1); and, 100% MLP, -0.76%($\pm$3.0). Correlational analyses demonstrated no significant relationships between performance time on the cycle ergometer and any hormonal response. The following significant correlations were observed: β-EP and capillary density (mm^{-2}) at 36% MLP(r=0.75), and cortisol with % type II fibers at 36% MLP(r=0.89).

Discussion

The exact mechanism(s) responsible for exercise-induced increases in β-EP, ACTH and cortisol remain unknown (3,16,23,26,41). Anaerobic exercise has been previously shown to be a potent stimulus for eliciting increases in plasma levels of these hormones (6,19,20,29,33). However, none of these studies have examined a complete range of anaerobic exercise intensities to further evaluate these responses.

With increases in plasma hormone levels occurring only at 36% MLP (107% $\dot{V}O_2$max), our data suggest there is not a linear response to increases in anaerobic exercise intensity. These data may also demonstrate a possible upper limit of response, beyond which a more intense anaerobic exercise stimulus does not perturbate peripheral plasma levels of these hormones. Whether this indicates a lack of post-translational processing or stimulation of preproopiomelanocortin at the gene level remains unknown (40).

These responses were neither time nor excerise dependent, as no significant relationships between plasma hormone concentrations and performance times or power outputs were observed. This suggests that a more complex interaction of variables is involved in producing the plasma elevations. Since the magnitude of increases observed for each hormone far exceeded changes that could be accounted for by plasma volume shifts, additional physiological mechanisms appear to be influencing these exercise responses.

The influence of anaerobic by-products or factors has been previously hypothesized by Farrell et al. (17) to provide a systemic stimulus for hypothalamic-pituitary-adrenal axis exercise activation. Relationships between lactic acid and concentrations of ACTH and cortisol have supported such an hypothesis (17,20). However, our data utilizing extremely high exercise intensities are inconsistent with this hypothesis. In this study significant increases in blood lactate were observed consequent to all four exercise intensities, while increases in β-EP, ACTH and cortisol were observed only following exercise at 36% MLP. The lack of any significant relationships between blood lactate and β-EP, ACTH or cortisol at these extremely high exercise intensities indicates that a different combination of stimuli or absence of certain stimuli, may be involved with hypothalamic-pitutiary-adrenal activation.

The influence of muscle fiber characteristics on hormonal responses of the hypothalamic-pituitary-adrenal axis has not been previously examined. Our results demonstrated two significant relationships. First, β-EP concentrations at 36% MLP were significantly related (r=0.75) to capillary

density (mm^{-2}) and, secondly cortisol concentrations at 36% MLP were significantly related (r=0.89) to % type II fibers. Although no cause-and-effect relationship can be implied, it is interesting to note that these relationships were found only when significant exercise-induced elevations took place. The possible mechanisms involved remain highly speculative. These data, however, may implicate some type of signal-feedback to the hypothalamus from opioid receptors on capillaries and cortisol receptors in type II muscle fibers which enhance release of corticotropin releasing factor (CRF). The stimuli involved and possible interactions of direct (i.e. neural) and indirect (i.e. blood borne) stimuli remain to be determined. It may be that the effectiveness of different feedback mechanisms is dependent upon the type and intensity of exercise utilized.

Consistent with other studies, our results demonstrated concomitant exercise-induced release of β-EP and ACTH with very high intensity exercise. The magnitude of the increases observed also were higher with anaerobic exercise (36% MLP) than previously reported responses to aerobic exercise (below $\dot{V}O_2max$). Still, it was consistent with brief short-term high intensity exercise elevations previously reported (6,19).

In this study, cortisol increases were observed at 15 min post-exercise at 36% MLP. The time course and magnitude of increase are almost identical to values reported in previous studies examining plasma cortisol responses to brief high intensity exercise (6,29,33). The temporal sequence in this study for ACTH and cortisol increases is also very similar to the study by Bruno et al.(6). These data appear to support the previous hypothesis that increases in plasma cortisol following brief high intensity exercise are in part due to ACTH-induced steroidogenesis (3,6).

In summary, the results of this study demonstrate that plasma responses of β-EP, ACTH and cortisol do not increase as the intensity of exercise is systematically increased at power productions in excess of maximal aerobic power. Our data at 36% MLP are consistent with previous studies which have demonstrated higher magnitudes of increase consequent to brief high intensity exercise compared to submaximal aerobic exercise stress. Still, not all high intensity exercise stress results in similar response patterns. Muscle fiber characteristics also appear to influence exercise-induced elevations of β-EP and cortisol. Further study is needed to continue to elucidate the mechanism(s) involved in plasma hormone responses to high intensity exercise.

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TABLE 1. Subject characteristics ($\bar{x} \pm$ 1SD) n=10

Age(yrs)	23.9 ± 3.8	Body Fat (%)	15.6 ± 5.4
Height(cm)	179.5 ± 5.4	Fat Free Mass (kg)	66.9 ± 6.3
Weight(kg)	79.5 ± 7.8	$\dot{V}O_2max$ (mL•Kg^{-1}•min^{-1})	46.2 ± 7.8

TABLE 2. Cardiorespiratory responses and performance characteristics for each exercise intensity [percentage of maximal leg power (MPL)].

	100% MLP	73%MLP	55%MLP	36%MLP	$\dot{V}O_2max$
Power (W)	848 (±128)	615 (±93)	461 (±70)	308 (±45)	264 (±34)
$\dot{V}O_2$ (L·min^{-1})	1.14 (±0.13)	1.69 (±0.48)	2.57 (±0.58)	3.45 (±0.56)	3.71 (±0.57)
Heart Rate (beats·min^{-1})	133 (±17)	155 (±14)	163 (±11)	173 (±8)	179 (±9)
Time (min)	0.10 *	0.261 (0.11)	0.768 (±0.31)	3.15 (±2.53)	3.38 (±0.59)

Values expressed as mean (± 1 SD)

*All subjects performed 5 revolutions at 60 rpm. Peak power was the highest of the 5 revolutions in this test.

TABLE 3. Muscle fiber characteristics of experimental subjects (n=10)

% Type II Fibers	52.4(± 8.8)
% Type IIA Fibers	42.2(± 7.3)
% Type IIB Fibers	10.2(± 8.4)
Type I area ($\mu m^2 \cdot 100$)	55.1(± 8.6)
Type II area ($\mu m^2 \cdot 100$)	62.6(± 13.2)
% Type II Area	55.2(± 13.2)
Capillary (mm^{-2})	275.5(± 62.5)
Capillary ($cap.fiber^{-1}$)	2.60(±0.66)

Values expressed as mean (± 1 SD)

Figure 1. Mean (±SE) plasma β-EP levels before and after (post) high intensity exercise at four different percentages of maximal leg power (MLP). *=significantly different (p<0.05) from resting baseline value.

Figure 2. Mean (±SE) plasma ACTH levels before and after (post) high intensity
exercise at four different percentages of maximal leg power (MLP).
*=significantly different (p<0.05) from resting baseline value.

Figure 3. Mean (±SE) plasma cortisol levels before and after (post) high
intensity exercise at four different percentages of maximal leg
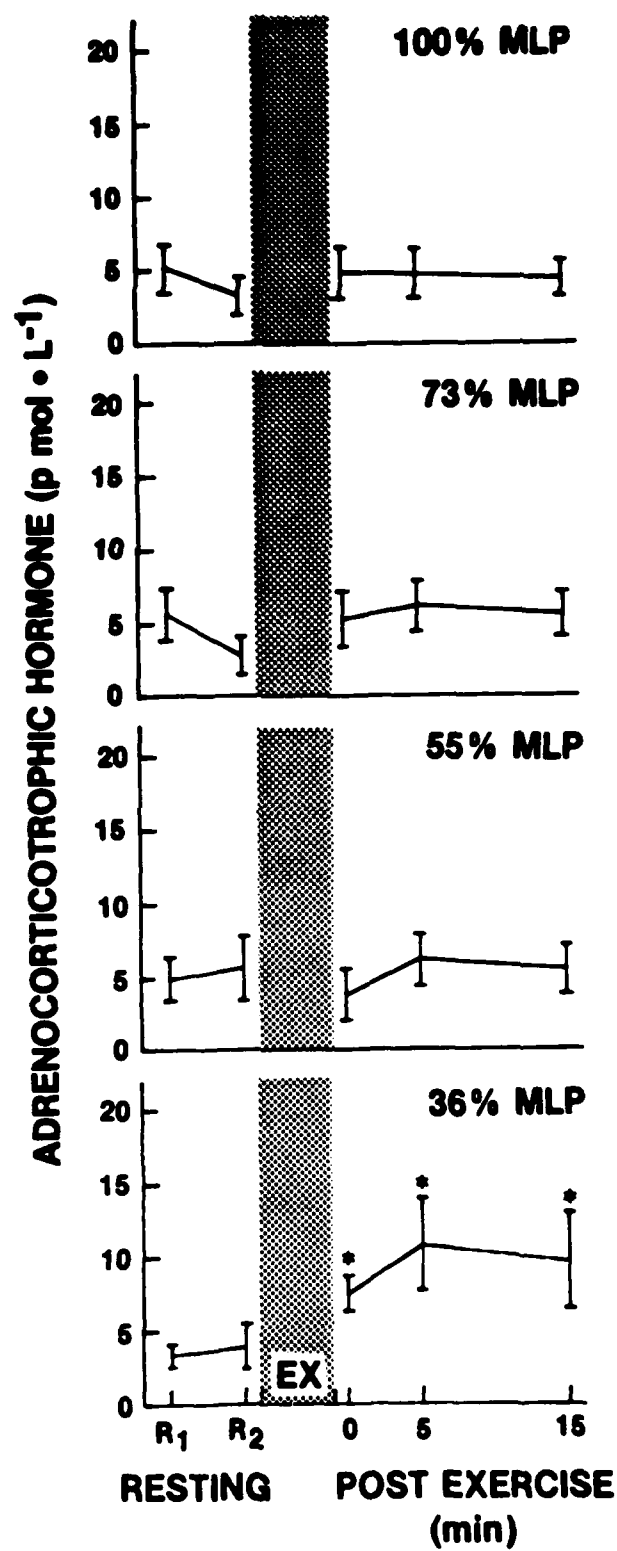power (MLP). *=significantly different (p<0.05) from resting
baseline value.

Figure 4. Mean (±SD) blood lactate levels before and after (post) high
intensity exercise at four different percentages of maximal leg
power (MLP). *=significantly different ($p < 0.05$) from resting
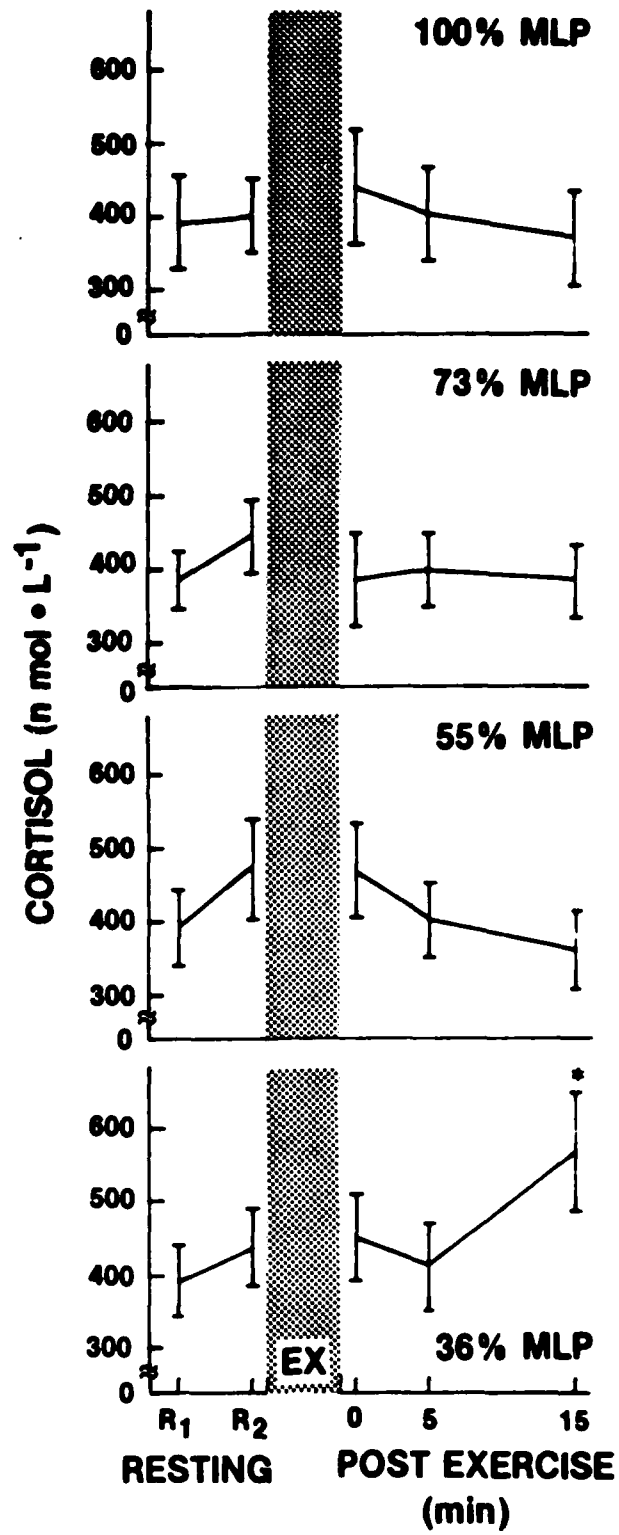baseline value.
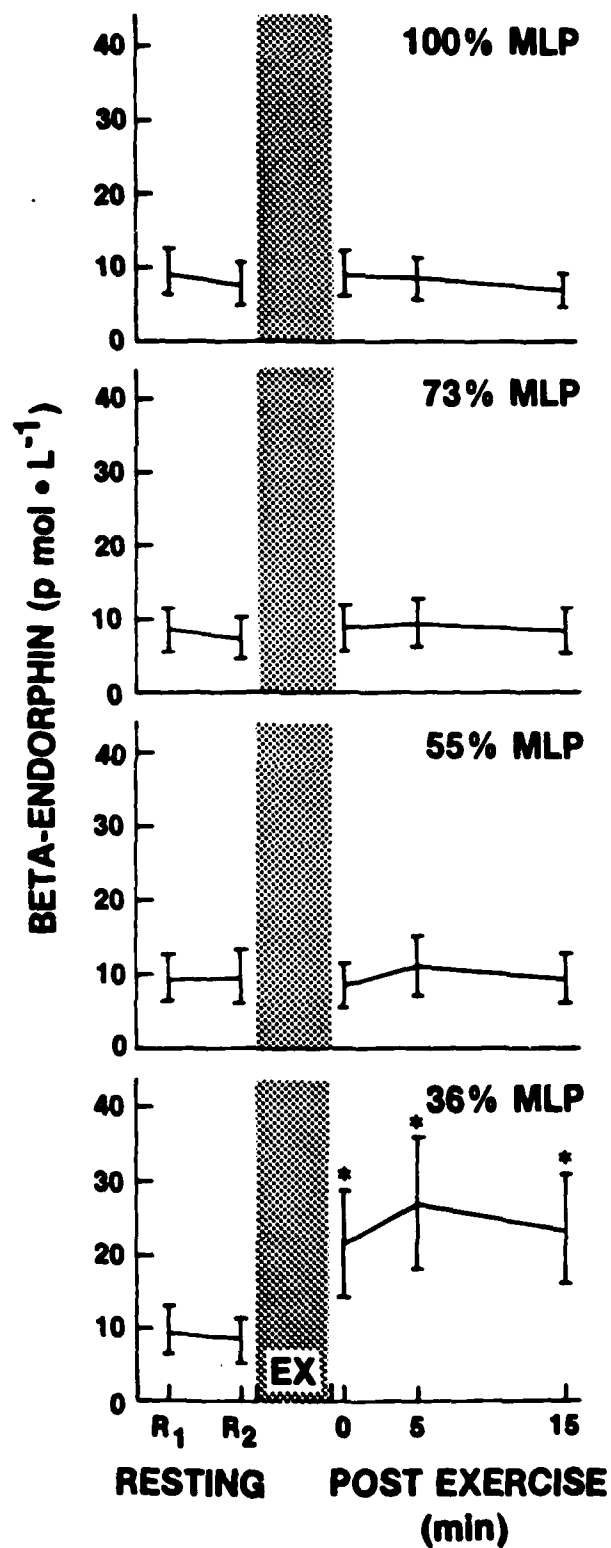
Fig 1

Fig 2

Fig 3
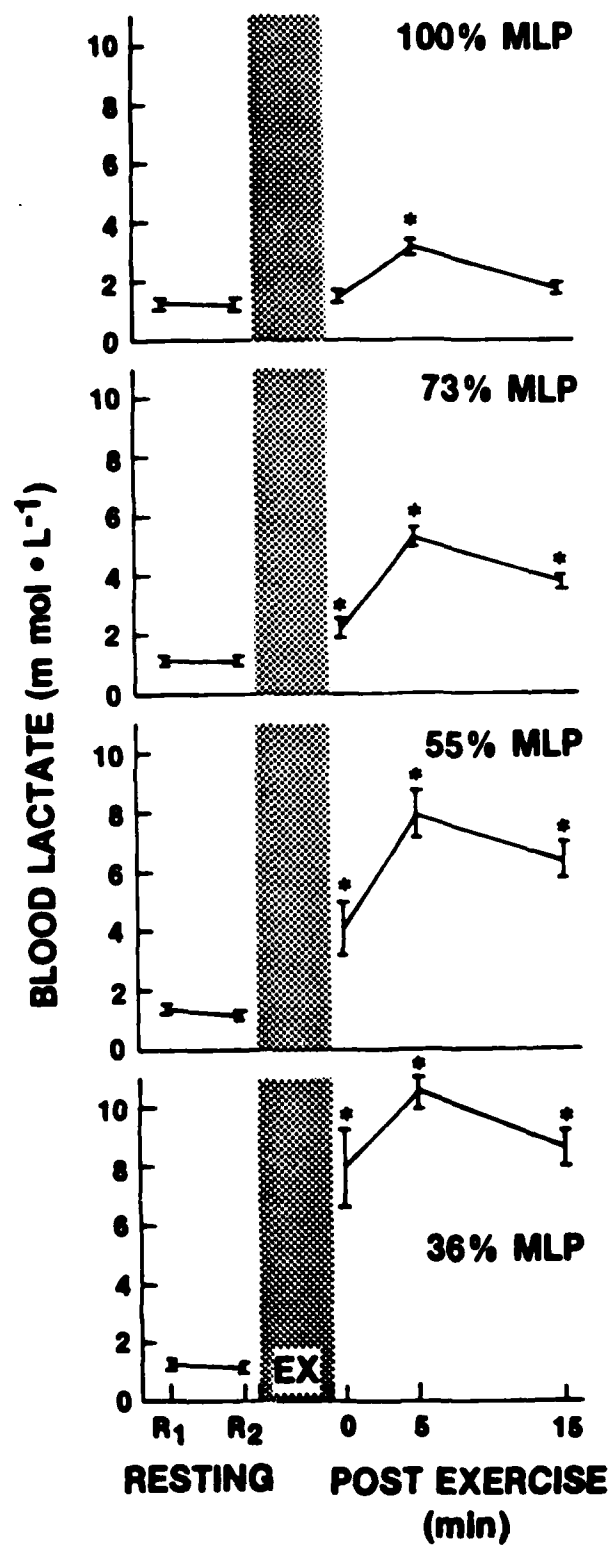
Fig 4

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